

Application No. 10/580,085
Amendment dated October 11, 2010
In Reply to Office Action dated June 9, 2010
Attorney Docket No. 4559-061539

AMENDMENTS TO THE SPECIFICATION

Please replace the second full paragraph on page 7 of the specification, beginning at line 21, with the following amended paragraph:

Site directed mutagenesis may be used to generate variants of seedy1 nucleic acids or portions thereof. Several methods are available to achieve site directed mutagenesis; the most common being PCR based methods (current protocols in molecular biology. Wiley Eds. <http://www.4ulr.com/products/currentprotocols/index.html>)
[<4ulr.com/products/currentprotocols/index.html>](http://www.4ulr.com/products/currentprotocols/index.html).

Please replace the first full paragraph on page 13 of the specification, from lines 6 to 25, with the following amended paragraph:

Methods for the search and identification of seedy1 homologues or DNA sequences encoding a seedy1 homologue, would be well within the realm of persons skilled in the art. Such methods, involve screening sequence databases with the sequences as provided by the present invention in SEQ ID NO 1 and 2 or 3 to 10, preferably a computer readable format of the nucleic acids of the present invention. This sequence information is available for example in public databases, that include but are not limited to Genbank (ncbi.nlm.nih.gov/web/Genbank) (<http://www.ncbi.nlm.nih.gov/web/Genbank>), the European Molecular Biology Laboratory Nucleic acid Database (EMBL) (ebi.ac.uk/ebi-docs/embl-db.html) (<http://www.ebi.ac.uk/ebi-docs/embl-db.html>) or versions thereof or the MIPS database (mips.gsf.de) (<http://mips.gsf.de>). Different search algorithms and software for the alignment and comparison of sequences are well known in the art. Such software includes GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percentage sequence identity and performs a statistical analysis of the similarity between the two sequences. The suite of programs referred to as BLAST programs has 5 different implementations: three designed for nucleotide sequence queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology: 76-80, 1994; Birren et al.,

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GenomeAnalysis, 1: 543, 1997). The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information.

Please replace the first full paragraph on page 14 of the specification, from lines 6 to 19, with the following amended paragraph:

The identification of domains or motifs, would also be well within the realm of a person skilled in the art and involves for example, a computer readable format of the nucleic acids of the present invention, the use of alignment software programs and the use of publicly available information on protein domains, conserved motifs and boxes. This protein domain information is available in the PRODOM (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html>) (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html>), PIR (<http://pir.georgetown.edu>) (<http://pir.georgetown.edu>) or pFAM (<http://pfam.wustl.edu>) (<http://pfam.wustl.edu>) database. Sequence analysis programs designed for motif searching may be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 3.0) can be found in the GCG package; or on the Internet site sdsc.edu/MEME/meme <http://www.sdse.edu/MEME/meme>. SIGNALSCAN version 4.0 information is available on the Internet site <http://biosci.cbs.umn.edu/software/sigscan.html>—<http://biosci.cbs.umn.edu/software/sigscan.html>. GENESCAN can be found on the Internet site genomic.stanford.edu/GENESCANW.html—<http://genomic.stanford.edu/GENESCANW.html>.

Please replace the paragraph bridging pages 14 and 15 of the specification, from page 14 line 28 to page 15 line 5, with the following amended paragraph:

Orthologues Orthologues in, for example, monocot plant species may easily be found by performing a so called reciprocal blast search. This may be done by a first blast involving blasting the sequence in question (for example, SEQ ID NO: 1 or SEQ ID NO: 2) against any sequence database, such as the publicly available NCBI database which may be found at: [ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) <http://www.ncbi.nlm.nih.gov>. If orthologues in rice were sought, the sequence in question would be blasted against, for example, the 28,469 full-length cDNA clones

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from *Oryza sativa* Nipponbare available at NCBI. BLASTn or tBLASTX may be used when starting from nucleotides or BLASTP or TBLASTN when starting from the protein, with standard default values. The blast results may be filtered. The full-length sequences of either the filtered results or the non-filtered results are then blasted back (second blast) against the sequences of the organism from which the sequence in question is derived. The results of the first and second blasts are then compared. An orthologue is found when the results of the second blast give as hits with the highest similarity a seedyl nucleic acid or protein; if one of the organisms is tobacco then a parologue is found. In the case of large families, ClustalW may be used, followed by a neighbour joining tree, to help visualize the clustering.

Please delete the paragraph on page 16, lines 1-10 and replace it with the following amended paragraph:

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in a protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)6-tag (SEQ ID NO: 19), glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

Please delete the paragraph on page 28, lines 35-37 and replace it with the following amended paragraph:

Figure 3 shows an N-terminal and C-terminal alignment of seedyl amino acids and deduced amino acids from ESTs, all from plants. This alignment was made with the program Align X of the VNTI software package. Motifs 1, 2, 3 and 4 are indicated with a bar. Figure 3, page 2 discloses "CDS0689" as residues 1-154 of SEQ ID NO: 2, "CDS0689 At" as residues 1-126 of SEQ ID NO: 12, "CDS0689 *Medicago trunculata*" as residues 1-88 of SEQ ID NO: 6, "CDS0689

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Os" as residues 1-89 of SEQ ID NO: 4, "CDS0689 Ta variant" as SEQ ID NO: 21, "CDS0689 So" as residues 1-85 of SEQ ID NO: 8 and the remaining sequences in Figure 3, page 2 as SEQ ID NOS 22-29, respectively, in order of appearance. Figure 3, page 3 discloses "CDS0689" as residues 339-475 of SEQ ID NO: 2, "CDS0689 At" as residues 294-402 of SEQ ID NO: 12, "CDS0689 *Medicago trunculata*" as residues 284-394 of SEQ ID NO: 6, "CDS0689 Os" as residues 297-431 of SEQ ID NO: 4 and the remaining sequences in Figure 3, page 3 as SEQ ID NOS 30-34, respectively, in order of appearance.

Please delete the paragraph on page 29, line 34 to page 30, line 5 and replace it with the following amended paragraph:

RNA extraction and cDNA synthesis.

Total RNA was prepared by using LiCl precipitation (Sambrook et al, 2001) and poly(A+) RNA was extracted from 500 mg of total RNA using Oligotex columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Starting from 1 mg of poly(A+) RNA, first-strand cDNA was synthesized by reverse transcription with a biotinylated oligo-dT25 primer (SEQ ID NO: 20) (Genset, Paris, France) and Superscript II (Life Technologies, Gaithersburg, MD). Second-strand synthesis was done by strand displacement with *Escherichia coli* ligase (Life Technologies), and DNA polymerase I (USB, Cleveland, OH) and RNase-H (USB).

Please replace the first full paragraph on page 31 of the specification, from lines 2 to 18, with the following amended paragraph:

A c-DNA library with average inserts of 1,400 bp was made with poly(A+) isolated from actively dividing, non-synchronized BY2 tobacco cells. These library-inserts were cloned in the vector pCMVSPORT6.0, comprising an attB gateway cassette (Life Technologies). From this library 46,000 clones were selected, arrayed in 384-well microtiter plates, and subsequently spotted in duplicate on nylon filters. The arrayed clones were screened by using pools of several hundreds of radioactively labelled tags as probe (among which the BY2-tag corresponding to the sequence CDS0689). Positive clones were isolated (among which the done reacting with the BY2-tag corresponding to the sequence CDS0689), sequenced, and aligned with the tag

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sequence. Alternatively, when the hybridization with the tag would fail, the full-length cDNA corresponding to the tag was selected by PCR amplification as follows. Tag-specific primers was designed using primer3 program [≤genome.wi.mit.edu/genome_software/other/primer3.html>](http://www.genome.wi.mit.edu/genome_software/other/primer3.html) (http://www.genome.wi.mit.edu/genome_software/other/primer3.html) and used in combination with the common vector primer to amplify partial cDNA inserts. Pools of DNA from 50.000, 100.000, 150.000, and 300.000 cDNA clones were used as templates in the PCR amplifications. Amplification product were isolated from agarose gels, cloned, sequenced and aligned with tags. The vector comprising the sequence CDS0689 and obtained as described above, was referred to as entry clone.

After page 34 of the specification, please substitute the accompanying Sequence Listing for the Sequence Listing pending with the application as filed.